Chapter 5

Microfluidic Chips Designed for Measuring Biomolecules Through a Microbead-Based Quantum Dot Fluorescence Assay

Kwang-Seok Yun, Dohoon Lee, Hak-Sung Kim, and Euisik Yoon

Summary

This chapter introduces the demonstration of specific antibody detection by using a microbead-based assay with quantum dot (QD) fluorescence on a polydimethylsiloxane (PDMS) microfluidic chip. The microfluidic chip is designed to isolate a single microbead where the binding reaction of antibodies occurs on the surface. The microfluidic chip is fabricated on a glass substrate using a transparent silicone elastomer, PDMS, for easy access of monitoring and flexible gate operations to capture the single microbead. For antibody detection, a sequence of functionalized assays has been performed in the fabricated chip, including the capturing of microbeads, antibody injection into a microchamber, quantum dot injection, and fluorescence detection. Various concentrations of human IgG antibodies have been introduced to bind to a single microbead captured and isolated inside a designated microchamber in a small volume of 75 pL. Fluorescence detection is monitored using a CCD camera after the second binding with the QDs conjugated with anti-human IgG. In this experiment, a human IgG antibody concentration below 0.1 μg/mL has been successfully detected.

Key words: Microbeads, Quantum dot, Microfluidics, Biomolecules, MEMS, Single microbead, Human IgG

1. Introduction

The demand for microfluidics is increasing rapidly in the fields of miniaturized chemical analysis systems, micro total analysis systems (μ-TAS), embedded medical devices, microdosing systems, and miniaturized production systems. Current micromachining and microelectromechanical systems (MEMS) technologies

James Weifu Lee and Robert S. Foote (eds.), *Micro and Nano Technologies in Bioanalysis*,
Methods in Molecular Biology, vol. 544
DOI 10.1007/978-1-59745-483-4_5,

have made it possible to implement the microfluidic functions on microchips, which are generally referred to as microfluidic chips. One of the most interesting applications of microfluidic chips is found in the detection of biomolecules because the analysis using microfluidic chips generally provides low cost, high throughput, fast analysis, and high sensitivity, so that it can be eventually implemented in micro portable systems *(1, 2)*.

Microfluidic chips are classified into two types according to their method of handling fluids on the chip. First, in droplet-based microfluidic systems, all of the fluids, including analytes, reagents, and buffer solution, are manipulated in a droplet form, being separated from each other by air or oil *(3, 4)*. This system can be imagined as a miniaturized method of the conventional analysis procedure normally done in a biological laboratory where the solutions are prepared, processed, and analyzed in glassware or microtubes. In the droplet-based microfluidic chip, a small droplet (normally from several tens of nanoliters to several microliters) substitutes for the whole solution in a glassware container *(3)*. Second, in continuous microfluidic systems, the fluids are manipulated in continuous streams. The different solutions are not isolated physically but make direct contact with each other, which causes unwanted mixing of solutions at the boundary. At the microscale, however, the mixing effects are generally negligible because the fluid stream occurs as laminar flow where little chaotic mixing is observed *(5, 6)*.

Bioanalytical methods using microbeads have been reported by many research groups because of the ease of modifying microbead surfaces for specific binding and of manipulation of the microbeads inside the microfluidic channels. The manipulations of microbeads have been achieved both in droplet-based microfluidic systems *(7)* and in continuous microfluidic systems *(8–15)*. The recent advancement of microfluidic technology has introduced a few methods of manipulating a single microbead on the microfluidic chip *(16–18)*. Yun et al. have reported on a passive manipulation of single microbeads in the microfluidic chip based on continuous microfluidics. A microbead can be passively manipulated in a fluid stream and positioned in a predetermined target microwell *(17)*. Medoro et al. used an active control method to manipulate cells (or microbeads) by using dielectrophoresis *(16, 18)*. This active control method has an advantage of providing high flexibility in bead/cell manipulation. However, it requires a complicated layout of control signals in chip implementation. This chapter describes a microfluidic chip for bioassay that allows the manipulation of microbeads down to single-bead resolution by using simple pneumatic control of a micro gate, without using complicated active manipulations.

1.1. Bioassay Using Quantum Dot Fluorescence

Quantum dots (QDs) are tiny nanocrystals that glow when stimulated by an external source such as ultraviolet (UV) light. Their size is determined by the number of atoms included in the QDs and determines their specific physical and optical properties, such as the color of light emitted. The possible applications of QDs include medical applications, lighting such as high-resolution television screens or flat panel displays, and quantum computers of the future. Especially, in optical detection for bioanalytical assays, QDs have many advantages as fluorescent reporters compared with conventional organic dyes.

The QDs provide detection results that are more sensitive because of the brighter luminescence resulting from their high extinction coefficients and quantum yields. The emission spectra of QDs can be modulated according to particle size. Therefore, simultaneous excitation of QDs in different sizes can be used for identifying various biomolecules with different emission colors, owing to their broad excitation spectra. In addition, the narrow emission bandwidth reduces interference in the detection spectrum. Because of their low sensitivity to photobleaching, QDs are optically more stable in long-term measurements. In addition, QDs can be well used in in vivo measurements because they can be easily transferred into cells after specific chemical modifications of the surfaces of the QDs. The toxicity to cells is known to be insignificant if the cells are exposed to QDs in appropriate duration *(19–21)*.

With these advantages, QDs have been widely used for the detection of biomolecules *(15, 22–32)*. QD-based Western blot technology was introduced by Bakalova et al. *(25, 26)*. Sun et al. used QDs modified with anti-human IgG to detect human IgG antibodies on protein microarrays *(27)*, reporting a detection limit of 2 μg/mL using a laser confocal scanner. Goldman et al. reported the detection of protein toxins (staphylococcal enterotoxin B, cholera toxin) by using QD-antibody conjugates *(28, 29)*. They detected the toxins coated on microtiter plates, and the lowest concentration of toxins that gave detectable signals over the background was approximately 15 ng/mL. In most cases, the detection of molecules has been performed on solid substrates such as well plates or glass plates after fixing the molecules on the surface.

This chapter introduces a QD-based bioassay using microbeads in a microfluidic chip. Use of the microfluidic chip is expected to result in high sensitivity because the efficiency of the chemical reaction will be improved by the continuous supply of reactants on the surface of the detection region—the surface of microbeads in this experiment. In the following sections, the sensitivity of single microbead-based bioassays using QD fluorescence on a microfluidic chip is discussed.

2. Materials

2.1. Chip Fabrication

1. *Substrate for mold formation*. Silicon wafers (Siltron, Gumi-si, South Korea), 4-in. diameter, single-side polished, test grade, 500-μm thick.
2. *Glass substrate*. Glass microscope slides (1 in. × 3 in.) (Corning, Corning, NY) are used as the substrate layers of the microfluidic chips.
3. *Mold structures*. SU-8 2005 and SU-8 2015 (MicroChem Corp., Newton, MA) for thin and thick structures, respectively. Store at a temperature of 4–21°C. Warm to room temperature before spin coating.
4. *Development*. SU-8 developer (MicroChem Corp.). Store at room temperature.
5. *Polydimethylsiloxane (PDMS)*. Sylgard 184 (Dow Corning, Midland, MI). Store at room temperature.
6. *Surface modification*. The surface of mold structures are self-assembled monolayer (SAM)-coated with tridecafluoro-(1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technologies, Inc., Bristol, PA). Toxic and irritating gas.

2.2. Microbead-Based Assay

1. *Buffer*. Phosphate-buffered saline (PBS), pH 7.4. Composition: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4. Sterilize by filtration (0.45 μm). Store at room temperature.
2. *Surface coating of microchannel*. 0.5% bovine serum albumin (BSA) in PBS. Store at 4°C.
3. *Reactive biotin*. EZ-Link® sulfo-NHS-LC-biotin (Pierce Biotechnology, Inc., Rockford, IL). Store desiccated at –20°C.
4. *Column*. Excess biotins are removed using D-Salt™ dextran desalting column (MW cutoff = 5 kDa, Pierce Biotechnology Inc.)
5. *Modified microbeads*. ProActive® streptavidin-coated microspheres (Bangs Laboratories, Inc., Fishers, IN), 10 μm in diameter. Store at 4°C.
6. *Quantum dot*. Qdot® 605 goat $F(ab')_2$ anti-human IgG conjugate (H + L) (Qdot, Invitrogen, Carlsbad, CA). Emission at 605 nm. Store at 4°C.

3. Methods

3.1. Fabrication of Microfluidic Chips

3.1.1. Design

Figure 1 shows the schematic view and operation of the proposed microfluidic chip for single microbead bioassay by exploiting QDs for enhanced fluorescence markers. The chip is composed of a microchamber in which a single microbead can be isolated by gate

operation. The two gates located on both sides of a microchamber are operated by pneumatic pressure applied in each gate control channel. These gates are designed to be partially open with a small gap at the initial phase when there is no pressure applied to the gate control channel. The gap is small enough to block the microbead but large enough to allow liquid or reagent to flow. When negative pneumatic pressure (or vacuum pressure) is applied to the control channel, the gate is fully opened and microbeads can be introduced into the microchamber. Initially the inlet gate is closed (or partially open) to prohibit any microbeads from entering into the microchamber. To capture a single microbead, the diluted solution containing a small number of microbeads is introduced through the inlet **(Fig. 1a)**. Next, the inlet gate is opened by applying negative (or vacuum) pressure to the upper gate control channel **(Fig. 1b)**. After a microbead enters the microchamber, the gate is closed to capture the microbead. Finally, the microbeads remaining outside of the chamber are washed away by reversing the flow direction from outlet to inlet **(Fig. 1c)**.

3.1.2. Fabrication

The microfluidic chip has been implemented using two polydimethylsiloxane (PDMS) layers and a glass substrate that are bonded together. **Figure 2** shows the fabrication procedure,

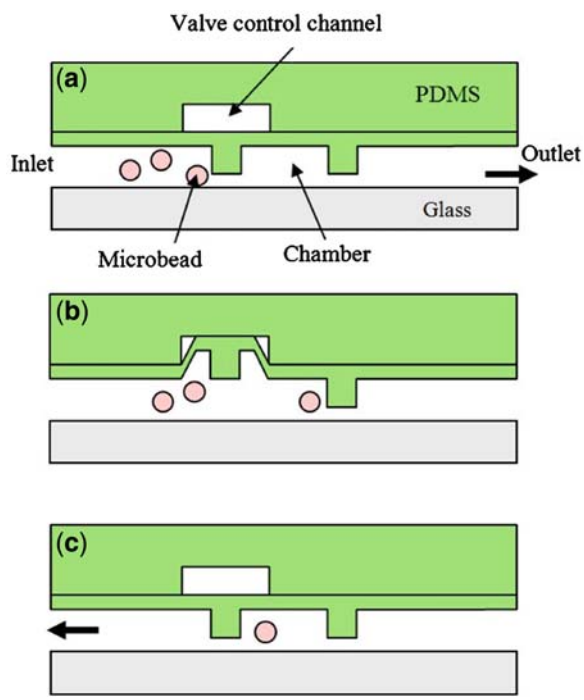

Fig. 1. Schematic view of each stage for the capturing of a single microbead. **(a)** Introduction of the beads; **(b)** valve open; and **(c)** valve closed/single bead capturing. (Reproduced from ref. *32* with permission from IOP).

which is composed of fabrication of the upper PDMS structure, fabrication of the lower PDMS layer, and the assembly process.

1. Fabrication of upper PDMS layer
   (a) Formation of PDMS replica molds **(Fig. 2a)**: A photosensitive epoxy, SU-8 (MicroChem Corp.), is patterned on silicon substrate to be used as a replica mold for PDMS. Next, the self-assembled monolayer (SAM) of tridecafluoro-(1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technology, Inc.) is coated on both the silicon and SU-8 surfaces for easy peeling of the PDMS layer.
   (b) Application of PDMS **(Fig. 2b)**: The PDMS prepolymer (Sylgard 184, Dow Corning) is poured onto the mold structure prepared in step *(1)* (*see* **Note 1**).
   (c) Peeling off PDMS **(Fig. 2c)**: The PDMS is cured at 80°C for 1 h and peeled off the substrate mold (*see* **Note 2**).
2. Fabrication of lower PDMS layer
   (a) Formation of PDMS replica molds **(Fig. 2d)**: SU-8 is coated with a thickness of 5 μm and exposed to ultraviolet light (UV) to define the gate structure. Without developing the first-coated SU-8 layer, the second SU-8 layer (thickness of 30 μm) is spin coated and exposed to UV to define the channel regions. Then, the unexposed region of SU-8 is developed by forming a PDMS mold for the channel and gate structures.
   (b) Application of PDMS **(Fig. 2e)**: The thin PDMS prepolymer is spin coated with a thickness of 60 μm on the fabricated SU-8 mold, followed by curing in an oven.
3. Assembly
   (a) Bonding of upper and lower PDMS layers **(Fig. 2f)**: The fabricated upper and lower PDMS layers are bonded together after surface modification by using oxygen plasma (*see* **Note 3**).
   (b) Peeling off PDMS **(Fig. 2g)**: The bonded PDMS layer is peeled off the silicon substrate, and the access holes for sample inlet, outlet, and vacuum control are formed by manual punching.
   (c) Bonding of PDMS structure and glass substrate **(Fig. 2h)**: The completed PDMS structure is bonded with the glass substrate after surface treatment by oxygen plasma.

Figure 3 shows the fabricated microfluidic chip and its magnified view of a microchamber. The size of a microchamber is 50 μm × 50 μm and the height is 30 μm. The microchamber is located between two gate valves. The initial gap of the gate valve is 5 μm to block the microbeads (10 μm in diameter) while maintaining the flow of liquid. Platinum electrodes (bright areas in magnified view) are

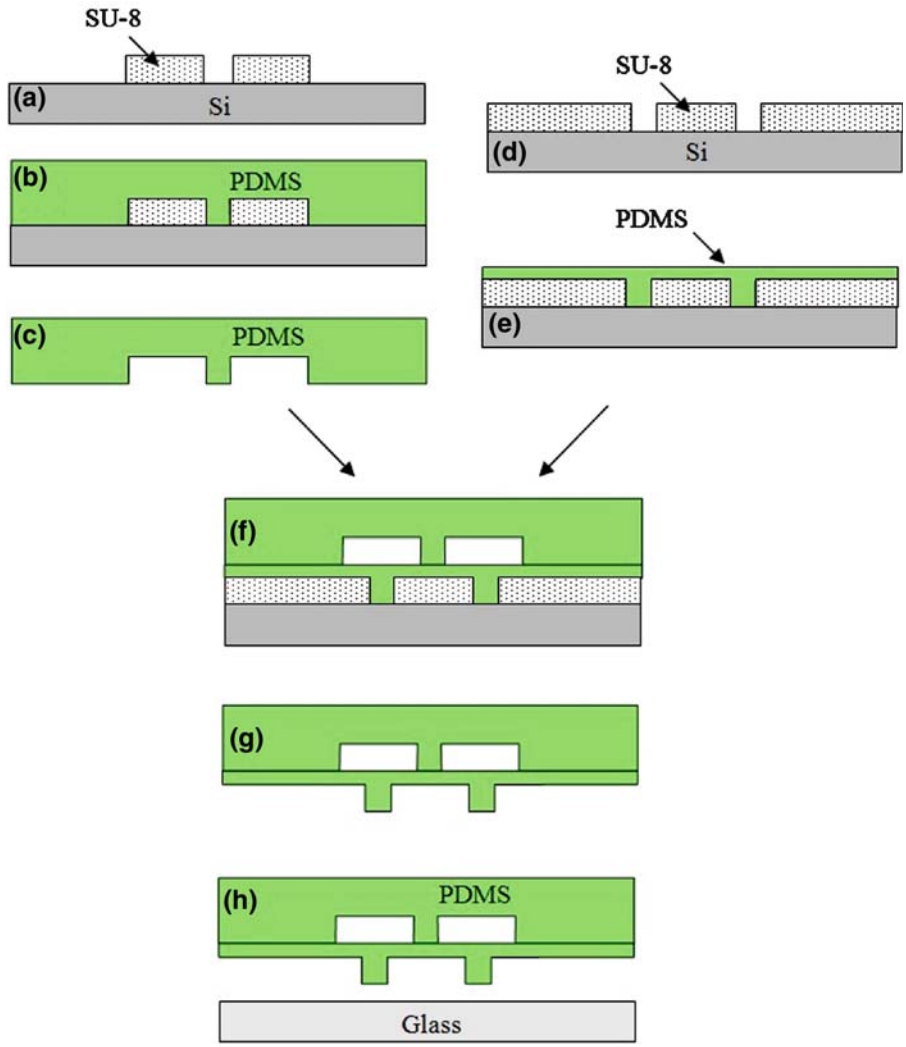

Fig. 2. Fabrication process. **(a)** SU-8 mold patterning for top PDMS plate; **(b)** PDMS pouring and curing; **(c)** peel off from mold; **(d)** SU-8 mold patterning for bottom PDMS membrane; **(e)** PDMS spin coating and curing; **(f)** bonding of top and bottom PDMS plates; **(g)** bonded PDMS layer peeled off from substrate; and **(h)** bonding with glass substrate. (Reproduced from ref.*32* with permission from IOP).

formed on the bottom surface of the microchannel and the microchamber for future applications.

3.2. Assay Using Single Microbeads on Microfluidic Chips

3.2.1. Preparation

The fabricated microfluidic chip is cleaned with ethanol, sterilized in a commercial autoclave, and fully dried in the oven (70°C) overnight (*see* **Note 4**). The surface of the microchannel is coated with 0.5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 2 h at room temperature to prevent nonspecific binding of proteins.

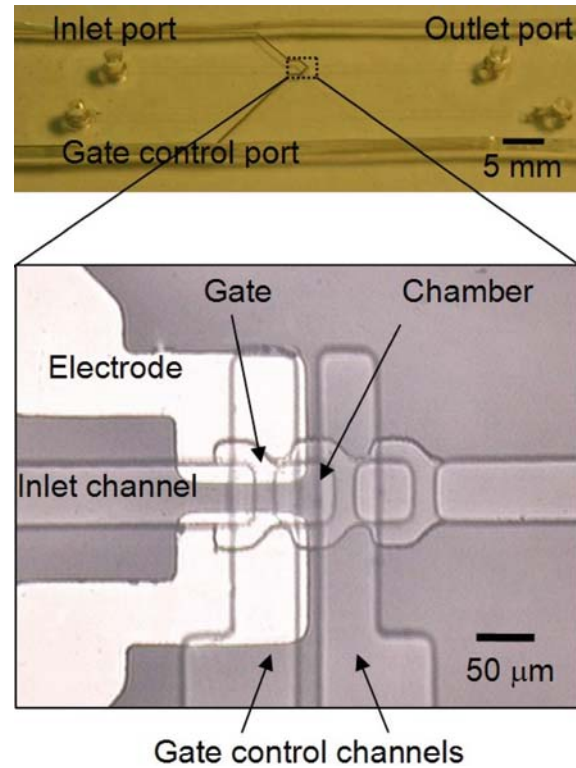

Fig. 3. Photograph of the fabricated microfluidic chip and magnified view. (Reproduced from ref.*32* with permission from IOP).

In this experiment, human IgG antibody is used to demonstrate the feasibility of QD fluorescent detection using single microbeads. The microbeads in this experiment are polystyrene microbeads (10 μm in diameter) coated with streptavidin (ProActive® streptavidin-coated microspheres, Bangs Laboratories, Inc.). For specific binding of human IgG, the surface of the microbead is coated with biotinylated protein A. The detailed procedures are as follows: sulfo-NHS-LC-biotin (Pierce Biotechnology Inc.) is added to protein A solution (10 mg/mL in PBS, 100 μL) at 12× molar excess. After 30 min reaction at room temperature, excess biotins are removed using a desalting column (Pierce Biotechnology Inc.). The biotin/protein molar ratio is determined to be ~4 by 4′-hydroxyazobenzene-2-carboxylic acid (HABA) assay (*see* **Note 5**). The biotinylated protein (b-protein A, ~56 μM) is divided into small aliquots and stored at −70°C until use. Before the immobilization of protein A, the microbeads are washed with PBS three times. The b-protein A (6 μM in PBS with 0.01% BSA and 0.05% Tween 20, 100 μL) is mixed with microbeads (1 mg) and allowed to bind to the surface of the microbeads by avidin-biotin interaction (overnight at 4°C) (*see* **Note 6**). After the binding reaction, the modified microbeads are washed with PBS three

times, resuspended in PBS (with 0.01% BSA and 0.05% Tween 20), and stored at 4°C (*see* **Note 7**).

The procedure for capturing a single microbead is as follows (refer to **Fig. 1**):

(a) The solution containing microbeads is deposited in the inlet port using a pipette (*see* **Notes 8** and **9**).

(b) The introduced microbeads are transferred to the inlet gate by applying suction at the outlet port using a microsyringe **(Fig. 1a)** (*see* **Notes 10** and **11**).

(c) When a microbead arrives at the inlet gate, the gate is opened to allow the microbead to pass through and then closed immediately, while keeping the outlet gate closed. This results in capturing and isolating a single microbead inside the microchamber **(Fig. 1b)** (*see* **Note 12**).

(d) After trapping the single microbead, the rest of the microbeads loaded in the microchannel are washed out by the reverse flow of PBS from the outlet to the inlet, as explained in the previous section **(Fig. 1c)** (*see* **Note 13**).

3.2.2. Detection of Protein

Figure 4 shows the experimental protocol for antibody detection using a microbead loaded in the microfluidic chip. All of the liquids, including PBS, antibody solution, and QD-conjugated secondary antibody solution, are introduced into the microfluidic chip through a silicone tube connected to the inlet port. To maintain a steady flow at a very low flow rate, all of the media and reagents are supplied at constant pressure by applying the same height difference of liquid levels between the inlet and outlet ports (*see* **Note 14**).

1. Experimental procedures

(a) Introduction of protein **(Fig. 4a)**: After a single microbead is isolated inside the microchamber, human IgG antibody is introduced through the inlet. To examine the detection limit of the single-bead assay, the experiment is conducted on five different microfluidic chips. Five different concentrations of human IgG are injected into each chamber for 30 min. The injected concentrations are 0 μg/mL, 0.01 μg/mL, 0.1 μg/mL, 1 μg/mL, and 10 μg/mL, respectively. The injected antibody is attached to the surface of the microbead, which is coated with biotinylated protein A.

(b) Introduction of QDs **(Fig. 4b)**: First the microchamber is washed with PBS for 30 min. Then, a 5 nM solution of anti-human IgG conjugated with CdSe/ZnS QDs ($\lambda_{emission}$ = ~605 nm, Qdot Corp.) is injected into each microchamber for 30 min (5 nM is the concentration of QDs).

(c) Washing and detection **(Fig. 4c)**: The channels and microchambers are completely washed with PBS solution. The

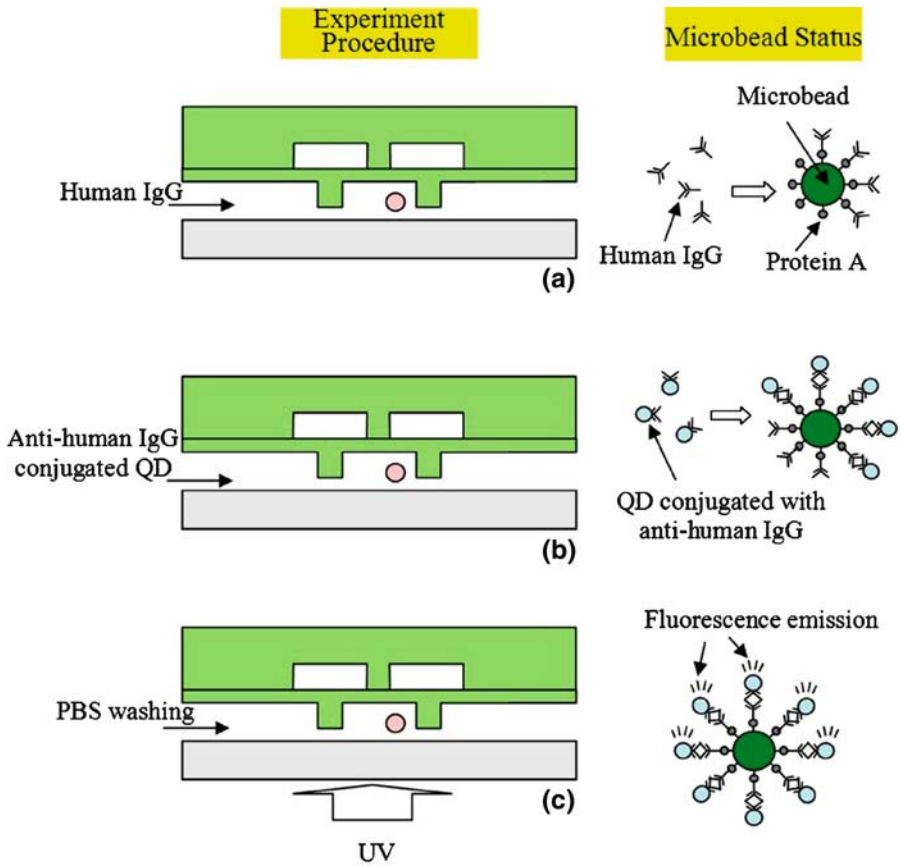

Fig. 4. The experimental procedure for microbead assay in a microchamber. **(a)** Human IgG injection after bead capturing; **(b)** anti-human IgG-conjugated QD injection after washing; and **(c)** washing with PBS and fluorescence detection. (Reproduced from ref. *32* with permission from IOP).

experimental results of this microbead-based assay are monitored using a fluorescence microscope (IX71, Olympus Co., Tokyo, Japan) with specialized filter set (XF304-2, Omega Optical, Inc., Brattleboro, VT) (*see* **Note 15**). The fluorescence images from microbeads are captured using a CCD camera and the average brightness is extracted using a graphics software (Photoshop, Adobe Systems, San Jose, CA).

3.3. Experimental Results

Figure 5 shows the experimental results presenting both the bright-field and the fluorescent images of the microchamber with a single microbead. The images are captured after the injection of human IgG followed by consequent introduction of QDs conjugated with anti-human IgG. The bright-field images show the successful isolation of a single microbead in each microchamber **(Fig. 5a)**. Although purified distilled water is used for the experiment, unknown substances sometimes flow into the microfluidic

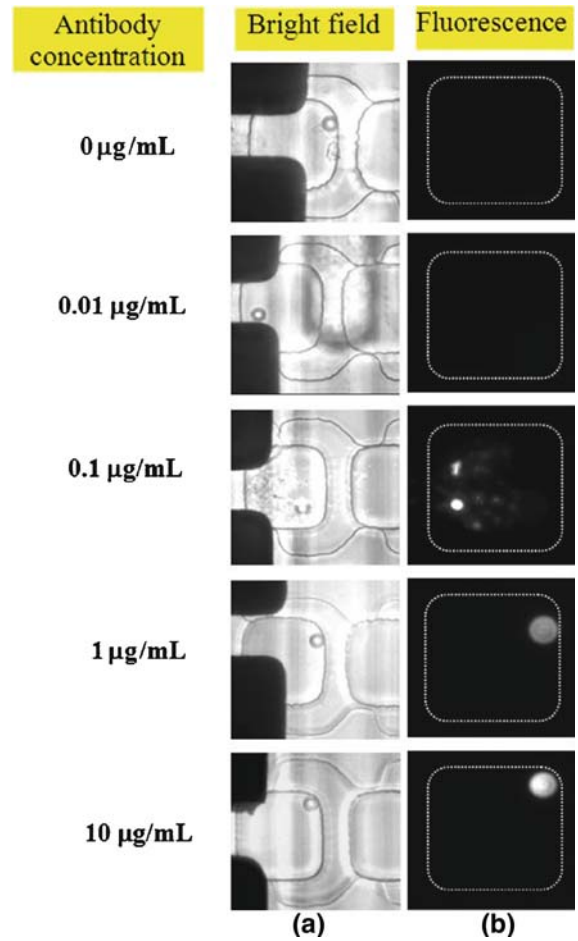

Fig. 5. Experimental results. **(a)** Bright field images of microchamber with a single microbead; and **(b)** fluorescence image according to each microbead in **(a)**. (Reproduced from ref.*32* with permission from IOP).

chip and obstruct the fluid stream, as shown in the 0.1-μg/mL case in this figure.

Figure 5b shows the experimental results as fluorescent images for various concentrations of antibody. The fluorescent image of the antibody concentration of 0 μg/mL verifies that the nonspecific binding of anti-human IgG (conjugated with QDs) to the bare microbead is negligible. The fluorescence signal can be detected when the antibody concentration becomes higher than 0.1 μg/mL and the intensity increases at higher concentrations. The normalized average intensities are plotted in **Fig. 6** as a function of antibody concentration. The deviation from the linear relationship at the concentration of 0.1 μg/mL may result from the reduced binding of antibody caused by the reduced flow rate of the reagent in the contaminated microchannel. The detection limit of this experiment is much lower than that of the previous

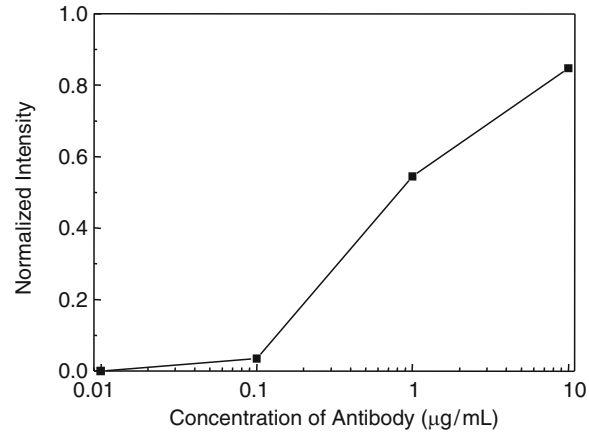

Fig. 6. Measured fluorescence intensity for various antibody concentrations. (Reproduced from ref. *32* with permission from IOP).

report, which was reviewed in **Subheading 1**. The detection limit in this experiment, 0.1 μg/mL, is approximately one order of magnitude lower than that reported in **ref**. *(27)*, in which human IgG antibodies are detected down to 2 μg/mL on a glass plate. In the microfluidic chip-based assay, binding efficiency can be improved because the reagents are continuously transferred to the surface of the microbeads, although this requires further experiments and analyses to be confirmed quantitatively. In addition, the commercial QD-conjugated anti-human IgG used in this experiment may have contributed to the increased sensitivity relative to that obtained using the QD reagents synthesized in **ref**. *(27)*.

4. Notes

1. In this step, after application of the PDMS solution onto the mold structure, the inherent bubbles are removed in a vacuum chamber.
2. The PDMS curing time can be reduced at higher temperature, but low curing temperature (<80°C) is recommended to prevent the thermal deformation of the mold structure made of SU-8.
3. To obtain the best bonding property, bonding should be completed in 5 min after surface modification.
4. The autoclave step is performed for 15 min at 120°C and 15 psi and the temperature of the drying oven is 70°C. These high-temperature sterilization and drying procedures do not disrupt the bonding between the PDMS layers and glass.

5. The assay was performed by following instructions provided by Pierce Biotechnology Inc. Generally, the addition of 12× molar excess sulfo-NHS-LC-biotin results in ~4 mol of conjugated biotin per mole of protein. Underderivatization or overderivatization can cause poor binding of biotinylated protein A to the microbead surface or a decrease in capture performance of the protein A.
6. Vigorous mechanical mixing with a stir bar should be avoided in this step, because it can damage the proteins. Mild mixing using a rocking plate is recommended.
7. The modified microbeads can be stored under these conditions for 2–3 weeks without degrading performance.
8. Before introducing microbeads into the microfluidic chip, the channel should be first filled with phosphate-buffered saline (PBS) to prevent the trapping of air in the microchannel.
9. To prevent a large number of microbeads from entering and clogging the microfluidic channel, diluted solution (~10^4/mL) is used in the experiment.
10. This step, as well as the following steps, should be done under microscope observation.
11. To transfer the microbeads to an inlet gate, the suction operation at the outlet port should be used instead of pushing the fluid to the inlet port. The pushing operation can generate pressure buildup inside the microchannel and opens the gates. This may result in failure of capturing microbeads.
12. The gate manipulation is manually performed with a syringe connected to the gate control channel through a silicone tube.
13. Again, in this step, suction at the inlet port is used.
14. The reservoir containing the injection liquid is placed approximately 40 cm higher than the outlet, which creates a constant pressure of about 4 kPa, making a continuous flow rate of 2 μL/min in the microfluidic chip.
15. The wavelength of excitation light was 470 nm and the emission wavelength from QDs was approximately 605 nm.

Acknowledgments

The authors acknowledge the financial support from the Intelligent Microsystems Program (IMP) of KIST under the "21C Frontier R&D program" and a research grant from Sogang University in 2007.

References

1. Manz, A. and Bekker, H. (1998). Microsystem Technology in Chemistry and Life Science (Springer Topics in Current Chemistry, Vol. 194), Springer, Berlin.
2. Hong, J. W. and Quake, S. R. (2003). Integrated nanoliter systems. *Nat. Biotechnol.* **21**, 1179.
3. Cho, S. K., Moon, H. and Kim, C.-J. (2003). Creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation for digital microfluidic circuits. *J. Microelectromechanical Systems* **12**, 70–80.
4. Wheeler, A. R., Kim, C.-J., Loo, J. A. and Garrell, R. L. (2004). Electrowetting-based microfluidics for analysis of peptides and proteins by matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* **76**, 4833–4838.
5. Thorsen, T., Maerkl, S. J. and Quake, S. R. (2002). Microfluidic large scale integration. *Science* **298**, 580–584.
6. Koch, M., Evans, A. and Brunnschweiler, A. (ed.) (2000). Microfluidic Technology and Applications. RSP, Baldock, Hertfordshire, England, pp. 227–237.
7. Shah, G. J., Pierstorff, E., Ho, D. and Kim, C.-J. (2007). Meniscus-assisted magnetic bead trapping on ewod-based digital microfluidics for specific protein localization. *Solid-State Sensors, Actuators and Microsystems Conference (TRANSDUCERS) 2007*, 707–710.
8. Lettieri, G.-L., Dodge, A., Boer, G., de Rooij, N. F. and Verpoorte, E. (2003). A novel microfluidic concept for bioanalysis using freely moving beads trapped in recirculating flows. *Lab Chip* **3**, 34– 39.
9. Verpoorte, E. (2003). Beads and chips: new recipes for analysis. *Lab Chip* **3**, 60N-68N.
10. Choi, J.-W., Oh, K. W., Thomas, J. H., Heineman, W. R., Brian Halsall, H., Nevin, J. H., Helmicki, A. J., Henderson, H. T., Ahn, C. H. (2002). An integrated microfluidic biochemical detection system for protein analysis with magnetic bead-based sampling capabilities. *Lab Chip* **2**, 27–30.
11. Sato, K., Tokeshi, M., Kimura, H., Kitamori, T (2001). Determination of carcinoembryonic antigen in human sera by integrated bead-bed immunoasay in a microchip for cancer diagnosis. *Anal. Chem.* **73**, 1213–1218.
12. Thomas, J. H., Kim, S. K., Hesketh, P. J., Halsall, H. B. and Heineman, W. R. (2004). Bead-based electrochemical immunoassay for bacteriophage MS2. *Anal. Chem.* **76**, 2700–2707.
13. Buranda, T., Huang, J., Perez-Luna, V. H., Schreyer, B., Sklar, L. A. and Lopez, G. P. (2002). Biomolecular recognition on well-characterized beads packed in microfluidic channels. *Anal. Chem.* **74**, 1149–1156.
14. Ali, M. F., Kirby, R., Goodey, A. P., Rodriguez, M. D., Ellington, A. D., Neikirk, D. P. and McDevitt, J. T. (2003). DNA hybridization and discrimination of single-nucleotide mismatches using chip-based microbead arrays. *Anal. Chem.* **75**, 4732–4739.
15. Gao, X. and Nie, S. (2004). Quantum dot-encoded mesoporous beads with high brightness and uniformity: rapid readout using flow cytometry. *Anal. Chem.* **76**, 2406–2410.
16. Medoro, G., Manaresi, N., Leonardi, A., Altomare, L., Tartagni, M. and Guerrieri, R. (2003). A lab-on-a-chip for cell detection and manipulation. *IEEE Sensors J.* **3**, 317–24.
17. Yun, K.-S. and Yoon, E, (2005). Micro/nanofluidic device for single-cell-based assay. *Biomed. Microdevices* **7**, 35–40.
18. Kim, B.-G., Yun, K.-S. and Yoon, E. (2005). Active positioning control of single cell/microbead in a micro-well array chip by dielectrophoresis. *Technical Digest of IEEE Int. Conf. on MEMS*, pp. 702–705.
19. Hoshino, A., Fujioka, K., Oku, T., Suga, M., Sasaki, Y. F., Ohta, T., Yasuhara, M., Suzuki, K. and Yamamoto, K. (2004). Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. *Nano Lett.* **4**, 2163–2169.
20. Chen, F., and Gerion, D. (2004). Fluorescent CdSe/ZnS nanocrystal-peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells. *Nano Lett.* **4**, 1827–1832.
21. Derfus, A. M., Chan, W. C. W. and Bhatia, S. N. (2004). Probing the cytotoxicity of semiconductor quantum dots. *Nano Lett.* **4**, 11–18.
22. Bruchez, M., Moronne, M., Gin, P., Weiss, S. and Alivisatos, A. P. (1998). Semiconductor nanocrystals as fluorescent biological labels. *Science* **281**, 2013–2016.
23. Chan, C. W. and Nie, S. (1998). Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* **281**, 2016–2018.
24. Yeh, H. C., Simone, E., Zhang, C. and Wang, T.-H. (2004). Single bio-molecule detection with quantum dots in a microchannel. *IEEE Int. Conf. on MEMS*, pp. 371–374.
25. Bakalova, R., Zhelev, Z., Ohba, H. and Baba, Y. (2005). Quantum dot-based western blot technology for ultrasensitive detection of tracer proteins. *J. Am. Chem. Soc.* **127**, 9328–9329
26. Zhelev, Z., Bakalova, R., Ohba, H., Imai, Y. and Baba, Y. (2006). Uncoated, broad fluorescent,

and size-homogeneous CdSe quantum dots for bioanalyses. *Anal. Chem.* **78**, 321–330.

27. Sun, B., Xie, W., Yi, G., Chen, D., Zhou, Y. and Cheng, J. (2001). Microminiaturized immunoassays using quantum dots as fluorescent label by laser confocal scanning fluorescence detection. *J. Immunol. Methods* **129**, 85–89.
28. Goldman, E. R., Balighian, E. D., Mattoussi, H., Kuno, M. K., Mauro, J. M., Tran, P. T. and Anderson, G. P. (2002). Avidin: a natural bridge for quantum dot antibody conjugates. *J. Am. Chem. Soc.* **124**, 6378–6382.
29. Goldman, E. R., Anderson, G. P., Tran, P. T., Mattoussi, H., Charles, P. T. and Mauro, J. M. (2002). Conjugation of luminescent quantum dots with antibodies using an engineered adaptor protein to provide new reagents for fluoroimmunoassays. *Anal. Chem.* **74**, 841–847.
30. Goldman, E. R., Clapp, A. R., Anderson, G. P., Uyeda, H. T., Mauro, J. M., Medintz, I. L. and Mattoussi, H. (2004). Multiplexed toxin analysis using four colors of quantum dot fluororeagents. *Anal. Chem.* **76**, 684–688.
31. Yun, K.-S., Lee, D., Kim, M. S., Kim, H.-S., Lee, G. M. and Yoon, E. (2004). High-throughput bio-molecule detection using microbead-based assay with quantum dot fluorescence in a microfluidic chip. *Proceedings of the International Conference on Miniaturized Systems for Chemistry and Life Sciences (Micro TAS'04)*, pp. 222–224.
32. Yun, K.-S., Lee, D., Kim, H.-S. and Yoon, E. (2006). A microfluidic chip for measurement of bio molecules using microbead-based quantum dot fluorescence assay. *Meas. Sci. Technol.* **17**, 3178–3183.